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(54) HIGH MOLECULAR WEIGHT POLYMER-BASED PRODRUGS

AUF POLYMEREN MIT HOHEM MOLEKULARGEWICHT BASIERENDE MEDIKAMENTVORSTUFEN

PROMEDICAMENTS A BASE DE POLYMERE A POIDS MOLECULAIRE ELEVE

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Description

TECHNICAL FIELD

[0001] The present invention relates to water soluble prodrugs. In particular, the invention relates to the use of relatively high molecular weight non-antigenic polymers to prepare prodrugs.

BACKGROUND OF THE INVENTION

[0002] Over the years, several methods of administering biologically-effective materials to mammals have been proposed. Many medicinal agents are available as water-soluble salts and can be included in pharmaceutical formulations relatively easily. Problems arise when the desired medicinal is either insoluble in aqueous fluids or is rapidly degraded in vivo. Alkaloids are often especially difficult to solubilize.

[0003] For example, several methods have been suggested to overcome the problems associated with administering paclitaxel, (also known as Taxol®, Bristol-Myers Squibb Co. NY, NY), which is insoluble in water. Currently, paclitaxel is administrated in physical admixture with a non-aqueous vehicle, cremophor-EL. This formulation, however, has several drawbacks. Hypersensitivity reactions have been associated with the vehicle and intravenous administration of the agent with this vehicle is also slow and causes discomfort to the patient.

[0004] Several methods have been suggested to enhance the aqueous solubility of paclitaxel. See, for example, PCT WO 93/24476, U.S. Patent No. 5,362,831, and Nicolaou, et al. Angew. Chem. Int. Ed. Engl. (1994) 33, No. 15/16, pages 1583-1587. Preparing water-soluble prodrug versions has also been explored.

[0005] Prodrugs include chemical derivatives of a biologically-active parent compound which, upon administration, will eventually liberate the active parent compound in vivo. Use of prodrugs allows the artisan to modify the onset and/or duration of action in vivo. In addition, the use of prodrugs can modify the transportation, distribution or solubility of a drug in the body. Furthermore, prodrugs may reduce the toxicity and/or otherwise overcome difficulties encountered when administering pharmaceutical preparations.

[0006] A typical example in the preparation of prodrugs can involve conversion of alcohols or thioalcohols to either organic phosphates or estets. Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, Ed. (1980), the disclosure of which is incorporated by reference herein.

[0007] Prodrugs are often biologically inert or substantially inactive forms of the parent or active compound. The rate of release of the active drug is influenced by several factors including the rate of hydrolysis of the converted ester or other functionality.

[0008] Recently, polyethylene glycol (PEG) and related polyalkylene oxides (PAO's) have been suggested as possible adjuncts for the preparation of paclitaxel prodrugs. See PCT WO 93/24476 supra, for example. PEG has also been conjugated to proteins, peptides and enzymes to increase aqueous solubility and circulating life in vivo as well as reduce antigenicity. See, for example, U.S. Patent Nos. 5,298,643 and 5,321,095, both to Greenwald, et al. These latter two references disclose, inter alia, biologically-active conjugates having substantially hydrolysis-resistant bonds (linkages) between a polyalkylene oxide and the target moiety. Thus, long-lasting conjugates rather than prodrugs per se were prepared. In most situations, the average molecular weight of the polymer included in the conjugate was preferably about 5,000 daltons.

[0009] PCT WO 93/24476 discloses using an ester linkage to covalently bind paclitaxel to water-soluble polyethylene glycols and provide a prodrug. Applicants, however, have discovered that the ester linkages described therein provide $T_{1/2}$ for hydrolysis of greater than four days in aqueous environments. Thus, most of the conjugate is eliminated prior to hydrolysis being achieved in vivo. It would be preferable to provide an ester linkage which allows more rapid hydrolysis of the polymer-drug linkage in vivo so as to generate the parent drug compound more rapidly.

[0010] It has also been surprisingly found that when only one or two polymers of less than 10,000 molecular weight are conjugated to alkaloids and/or organic compounds, the resulting conjugates are rapidly eliminated in vivo. In fact, such conjugates are so rapidly cleared from the body that even if a hydrolysis-prone-ester linkage is used, not enough of the parent molecule is regenerated in vivo to make the PAO-drug conjugate worthwhile as a prodrug.

[0011] Ohya, et al., J. Bioactive and Compatible Polymers Vol. 10 Jan., 1995, 51-66, disclose doxorubicin-PEG conjugates which are prepared by linking the two substituents via various linkages including esters. The molecular weight of the PEG used, however, is only about 5,000 at best. Thus, the true in vivo benefits would not be realized because the conjugates would be substantially excreted prior to sufficient hydrolysis of the linkage to generate the parent molecules.

[0012] Yamaoka, et al. J. Pharmaceutical Sciences, Vol. 83, No. 4, April 1994, pages 601-606, disclose that the half-life of unmodified PEG in circulation of mice after IV administration extended from 18 minutes to one day when molecular weight was increased from 6,000 to 190,000. Yamaoka, et al., however, failed to consider the effect of linking the polymer to a drug would have on the drug. Also, Yamaoka, et al. failed to consider that aqueous solutions of higher

molecular weight polymers are quite viscous and difficult to dispense through the narrow-bore devices used to administer pharmaceutical preparations.

[0013] U.S. Patent No. 4,943,579 discloses the use of certain amino acid esters in their salt forms as water soluble prodrugs. The reference does not, however, disclose using the amino acids as part of a linkage which would attach relatively high molecular weight polymers in order to form prodrugs. As evidenced by the data provided in Table 2 of the '579 patent, hydrolysis is quick. At physiologic pH, the insoluble base is rapidly generated after injection, binds to proteins and is quickly eliminated from the body before therapeutic effect can be achieved.

[0014] WO 96/23794 discloses polymer-based prodrugs comprising a high molecular weight polyalkylene oxide moiety (having a molecular weight of from 20,000 to 80,000) and one or two drug moieties coupled to the polymer part by an ester-containing linker which may be derived from the amino acid glycine.

[0015] WO 95/11020 discloses 2'- and/or 7-substituted taxoids, wherein the substituent is a polyalkylene glycol coupled to the taxoid by a carbonate or ester-containing linker which may be derived from the amino acid glycine.

[0016] In summary, previous prodrugs based on conjugates of a parent drug compound and a water soluble polymer have not been successful for various reasons including excessively slow hydrolysis of the polymer from the parent drug and excessively rapid clearance of the prodrug from the body. In addition, improvements in prodrugs based on simple amino acid esters have been sought to overcome the rapid regeneration of the parent compound at physiological pH.

[0017] The present invention addresses the shortcomings described above.

SUMMARY OF THE INVENTION

[0018] In one aspect, the invention provides a prodrug compound having the formula (I) or (II):

$$D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}R^2 \quad (I)$$

$$[D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}]_2R^3 \quad (II)$$

wherein

D is a residue of a drug having an ester-forming hydroxyl group,

R^2 and R^3 are a residue of a water soluble polyalkylene oxide or activated polyalkylene oxide having a molecular weight of from 20,000 to 80,000, and

the moiety -CO-CH(R^1)-NH- is a residue of an (*l*) amino acid, a (*d*) amino acid or a mixture of (*l*) and (*d*) amino acid.

[0019] As is evident from the formulae (I) and (II), polymer-based mono- and bis-prodrugs are contemplated.

[0020] The prodrugs preferably include a water-soluble polyalkylene oxide polymer as R^2 or R^3. More preferably, the polymer is a polyethylene glycol and has a molecular weight of at least about 20,000.

[0021] In certain preferred aspects of the invention, the drug compound (designated D herein) attached to the polymer is a taxane such as paclitaxel or taxotere. In other aspects of the invention, the drug compound is camptothecin, etoposide, cis-platin derivatives containing OH groups, floxuridine or podophyllotoxin. In still further embodiments, other oncolytic agents, non-oncolytic agents such as anti-inflammatory agents, including steroidal compounds, as well as therapeutic low molecular weight peptides such as insulin are also contemplated.

[0022] One of the chief advantages of the compounds of the present invention is that the prodrugs achieve a proper balance between the rate of parent drug-polymer linkage hydrolysis and the rate of clearance of prodrug from the body. The linkage between the polymer and the parent compound, also referred to herein as a biologically-active nucleophile, hydrolyzes at a rate which allows a sufficient amount of the parent molecule to be released in vivo before clearance of the prodrug from the plasma or body.

[0023] Another advantage of the present invention is that in certain preferred embodiments, the prodrug compound includes a racemic mixture of the linker portion joining biologically active material linked to high molecular weight polymers using both the (d) and (l) forms of the prodrug linkage . This unique blend allows the artisan to design a novel prodrug complex having controlled release properties in which there is an initial relatively rapid release of the drug from the prodrug form, due to the relatively rapid enzymatic cleavage of the *(l)* forms of the amino acid linker portion, followed by a relatively slow release of the drug from the prodrug as a result of the hydrolysis of *(d)* form of the amino acid linker portion. Alternatively, the *(d)* and *(l)* forms of the amino acids can be used separately to employ the unique hydrolysis properties of each isomer, i.e. *(l)*- relatively rapid, *(d)*-slower hydrolysis. The compounds of the present invention are also designed to include polymers of adequate molecular weight to insure that the circulating life of the prodrugs is sufficient to allow the necessary amount of hydrolysis (and thus regeneration of therapeutic amounts of

the drug in vivo) before elimination of the drug. Stated in another way, the compounds of the present invention are preferably designed so that the circulating life $T_{1/2}$ is greater than the hydrolysis $T_{1/2}$.

[0024] Methods of making and using the compounds described herein are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025]

Figures 1 and 2 are schematic representations of the reactions carried out in accordance with Examples 1-8.
Figure 3 is a schematic representation of the reaction carried out in accordance with Example 9.
Figure 4 is a schematic representation of the reaction carried out in accordance with Example 10.
Figure 5 is a schematic representation of the reactions carried out in accordance with Examples 9b and 9c.

DETAILED DESCRIPTION OF THE INVENTION

A. THE PRODRUGS

[0026] In the invention, the prodrug compounds of the present invention contain hydrolyzable linkages between the polymer portion and a biologically active moiety derived from a biologically active moiety or nucleophile, i.e. native or unmodified drug. These linkages are ester linkages designed to hydrolyze at a rate which generates sufficient amounts of the biologically active parent compound in a suitable time period so that therapeutic levels of the parent therapeutic moiety or moieties are delivered prior to excretion from or inactivation by the body. The term "sufficient amounts" for purposes of the present invention shall mean an amount which achieves a therapeutic effect as such effect is understood by those of ordinary skill in the art.

[0027] In one preferred embodiment of the invention, the prodrug compound of the invention has the formula

$$D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}R^2 \quad (I)$$

wherein

D is a residue of a drug having an ester-forming hydroxyl group,

R^2 is a residue of a water soluble polyalkylene oxide or activated polyalkylene oxide having a molecular weight of from 20,000 to 80,000, and

the moiety -CO-CH(R^1)-NH- is a residue of an (*l*) amino acid, a (*d*) amino acid or a mixture of (*l*) and (*d*) amino acid.

[0028] Preferably, the polymer portion, designated R^2 herein, is further substituted with a terminal capping moiety (Z) which is distal to the primary linkage attaching D to the polymer. A non-limiting list of suitable capping groups includes OH, C_{1-4} alkyl moieties, and biologically active and inactive moieties.

[0029] In another preferred embodiment of the invention, the prodrug compound of the invention has the formula

$$[D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}]_2R^3 \quad (II)$$

wherein

D is a residue of a drug having an ester-forming hydroxyl group,

R^3 is a residue of a water soluble polyalkylene oxide or activated polyalkylene oxide having a molecular weight of from 20,000 to 80,000, and

the moiety -CO-CH(R^1)-NH- is a residue of an (*l*) amino acid, a (*d*) amino acid or a mixture of (*l*) and (*d*) amino acid.

[0030] In preferred aspects of the invention, the linkage attaching D to the polymer includes an amino acid ester spacer such as alanine or phenylalanine.

B. THE PRODRUG LINKAGE

1. The Amino Acid Portion of the Linker

[0031] As mentioned above in Section A, one aspect of the invention includes using an amino acid ester spacer such as alanine within the linkage attaching the polymer R_2 to the biologically active moiety D. This portion of the linkage can be attached to the D portion directly as illustrated in Figure 1 using t-Boc-*l* (or *d* or racemic)-alanine or by converting

a PEG acid or diacid with the *l*- or *d*-alanine-t-butyl ester as shown, for example, in Figure 2.

2. Hydrolysis and Parent Drug Regeneration

[0032] The prodrug compounds of the present invention are designed so that in plasma the $T_{1/2}$ circulation is greater than the $T_{1/2}$ hydrolysis, which in turn is greater than the $T_{1/2}$ for elimination, i.e.

$$T_{1/2} \text{ circulation} > T_{1/2} \text{ hydrolysis} > T_{1/2} \text{ elimination.}$$

[0033] The prior art had several shortcomings associated with its approach to providing polymer based prodrugs. For example, in some cases, the molecular weight of the polymer was insufficient, i.e. 10,000 Daltons or less, regardless of the linkage used to attach the parent drug to the polymer. In other cases, a polymer of sufficient molecular weight was proposed but the linkage was not designed to allow sufficient in vivo hydrolysis and release of the parent molecule. The compounds of the present invention overcome these shortcomings by including not only polymers of sufficient weight but also linkages which meet the criteria discussed above.

[0034] As preferred in the embodiment discussed above, the ester-based linkages included in the compounds have a $T_{1/2}$ hydrolysis in the plasma of the mammal being treated which is long enough to allow the parent compounds to be released prior to elimination. Some preferred compounds of the present invention have plasma $T_{1/2}$ hydrolysis rates ranging from about 30 minutes to about 12 hours. Preferably, the compounds have a plasma $T_{1/2}$ hydrolysis ranging from about 1 to about 8 hours and most preferably from about 2.5 to about 5.5 hours. The compounds thus provide a distinct advantage over the rapidly hydrolyzed prodrugs of the prior art, such as those described in U.S. Patent No. 4,943,579 which are all about 45 minutes or less and are of limited practical value. The parent compounds appear to be rapidly regenerated in vivo and quickly eliminated from circulation. While Applicants are not bound by theory, in those aspects of the invention where prodrugs are formed, regeneration of sufficient amounts of the parent compound during the time the prodrug remains in circulation is believed to be a key to providing an effective prodrug compound.

C. SUBSTANTIALLY NON-ANTIGENIC POLYMERS

[0035] The prodrug compounds of the present invention include a water-soluble polyalkylene oxide such as polyethylene glycols which are also preferably substantially non-antigenic. It will be understood that the water-soluble polyalkylene oxide will be functionalized for attachment to the alpha-amino group of the amino acid linker.

[0036] In particular, polyethylene glycols (PEG's), mono-activated, C_{1-4} alkyl-terminated PAO's such as mono-methyl-terminated polyethylene glycols (mPEG's) are preferred when mono- substituted polymers are desired; bis-activated polyethylene oxides are preferred when disubstituted prodrugs are desired. In order to provide the desired hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids are used. Suitable PAO acids can be synthesized by converting mPEG-OH to an ethyl ester. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487(1987) and Veronese, F.M., et al., J. Controlled Release 10; 145 (1989). Alternatively, the PAD-acid can be synthesized by converting mPEG-OH into a *t*-butyl ester. See, for example, commonly assigned U.S. Patent Application Serial No. 08/440,732 filed May 15, 1995. The disclosures of each of the foregoing are incorporated by reference herein.

[0037] Although PAO's and PEG's can vary substantially in molecular weight, polymers having molecular weight ranges of at least 20,000 are preferred. Polymers ranging from about 20,000 to about 80,000 are selected for the purposes of the present invention. Molecular weights of from about 25,000 to about 45,000 are preferred and 30,000 to about 42,000 are particularly preferred. The molecular weight of the polymer selected for inclusion in the prodrug must be sufficient so as to provide sufficient circulation of the prodrug during hydrolysis of the linker.

[0038] The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

[0039] As mentioned above, the prodrugs of the present invention include one or two equivalents of drug per equivalent of the polymer. As such, preferred polymers can be functionalized to form the bis-prodrugs when reacted with a sufficient amount of a parent compound.

[0040] Although, the prodrugs of the present invention can be formed using any of the substantially non-antigenic polymers described herein, the following polyalkylene oxide derivatives, i.e. PEG-acids and PEG-diacids are especially preferred for use in formation of the prodrug.

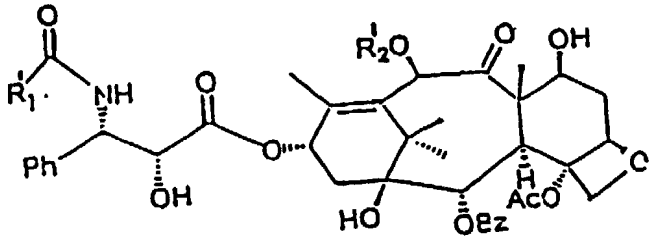
[0041] It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, POG acids, etc., as well as other bifunctional linking groups are also contemplated.

D. PRODRUG CANDIDATES

1. Taxanes and Taxane Derivatives

[0042] One class of compounds included in the prodrug compounds of the present invention is taxanes. For purposes of the present invention, the term "taxane" includes all compounds within the taxane family of terpenes. Thus, taxol (paclitaxel), 3'-substituted tert-butoxy-carbonyl-amine derivatives (taxoteres) and the like as well as other analogs available from, for example, Sigma Chemical of St. Louis, Missouri are within the scope of the present invention. Representative taxanes are shown below.

(IV)



Paclitaxel: $R_1' = C_6H_5$; $R_2' = CH_3CO$

Taxotere: $R_1' = (CH_3)_3CO$; $R_2' = H$

[0043] These compounds have been found to be effective anti-cancer agents. Numerous studies indicate that the agents have activity against several malignancies. To date, their use has been severely limited by, among other things, their short supply, poor water solubility and hypersensitivity. It is to be understood that other taxanes including the 7-aryl-carbamates and 7-carbazates disclosed in commonly assigned U.S. Patent Nos. 5,622,986 and 5,547,981 can also be included in the prodrugs of the present invention. The contents of the foregoing U.S. patents are incorporated herein by reference.

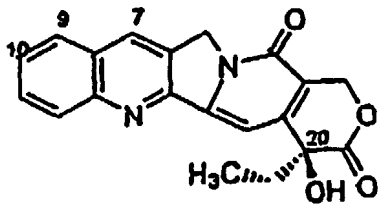
[0044] Although the examples describe inter alia paclitaxel for illustrative purposes, it is to be understood that the methods described herein are suitable for all taxanes and related molecules. The only limitation on this provision is that the selected taxanes must be capable of undergoing 2' position modifications described herein. Paclitaxel, however, is a preferred taxane.

[0045] Synthesis of the taxane-based prodrugs of the invention is set forth below in section E and in the Examples. In general, however, a taxane having the 2'-position available for substitution is reacted with a suitably activated polymer such as a PEG acid under conditions sufficient to cause the formation of a 2' ester linkage between the two substituents. The corresponding diester can be prepared by reacting at least about 2 equivalents of taxane per polymer diacid. Even when two equivalents of taxane are reacted with the polymer diacid, the resulting conjugate can contain minor amounts (i.e. up to 25%) by weight of a monoester species containing an acyl urea or carboxylic acid distal to the polymer-taxane linkage with regard to the polymer. These compositions are also capable of delivering a biological effect. It is preferred that the polymer acid have a molecular weight of at least about 20,000. See Figure 3 as illustrative example.

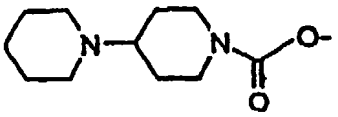
2. Camptothecin and Related Topoisomerase I Inhibitors

[0046] Camptothecin is a water-insoluble cytotoxic alkaloid produced by *camptoteca accuminata* trees indigenous to China and *nothapodytes foetida* trees indigenous to India. Camptothecin and related compounds and analogs are also known to be potential anticancer or antitumor agents and have been shown to exhibit these activities in vitro and in vivo. Camptothecin and related compounds are also candidates for conversion to the prodrugs of the present invention. See, for example, U.S. Patent No. 5,004,758 and Hawkins, Oncology, December 1992, pages 17-23. Camptothecin and related analogues have the structure:

(V)



20(S)-Camptothecin

Camptothecin analogues	Position 7	9	10
Topotecan	H	$CH_2N(CH_3)_2$	OH
CPT-11	$-CH_2-CH_3$	H	

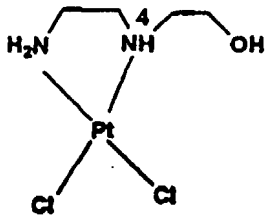
Additional camptothecin analogs include those reported in the literature including the 10-hydroxycamptothecins, 11-hydroxycamptothecins and/or 10,11-dihydroxycamptothecins, 7-and/or 9- alkyl, substituted alkyl, cycloalkyl, alkoxy, alkenyl, aminoalkyl, etc. camptothecins, A-ring substituted camptothecins such as 10,11-alkylenedioxycamptothecins, such as those disclosed in U.S. Patent No. 5,646,159, the contents of which are incorporated herein by reference, etc.

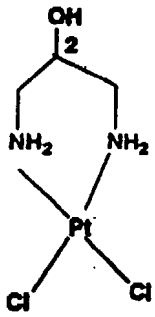
[0047] Formation of a monoester camptothecin prodrug can be accomplished by reacting one or more equivalents of a suitably (acid) activated polymer with one equivalent of the camptothecin derivative under conditions sufficient to effectively convert the 20- OH to an ester-linked polymeric based prodrug. Camptothecin diesters are similarly prepared by reacting at least about 2 and preferably greater equivalents of the camptothecin with a suitably prepared PAO diacid. Details concerning the reaction schemes and conditions are provided in Section E, below, Figure 1, and in the Examples.

[0048] In addition to the foregoing camptothecin analogs, it has been found that new 20(S)camptothecin-mono-PEG ester compounds can be formed when a diacid PEG is used with certain carbodiimide condensing agents with the appropriate stoichiometry. For example, the alpha terminus of the polymer is converted to a camptothecin-PEG ester and the omega terminus of the PEG diacid is converted from the acid to an acyl dialkyl urea, depending on the dialkyl carbodiimide employed to effect conjugation. These derivatives show antitumor activity in vivo and upon NMR inspection, cross-linking was found to be negligible. In most preferred aspects, however, bis- prodrug camptothecin compounds are formed by linking each of the alpha and omega termini of the polymer to the 20 S position of camptothecin when a carbodiimide is used as the condensing agent. In alternative aspects, higher amounts of the diester can be obtained by the use of a Mukaiyama reagent, i.e. 2-chloro-l-methylpyridinium iodide.

3. Additional Biologically-Active Moieties

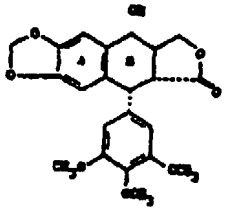
[0049] In addition to the foregoing molecules, the prodrug formulations of the present invention can be prepared using many other compounds. For example, biologically-active compounds such as cis-platin derivatives containing OH groups, i.e.





mono- and bis-PEG esters derived from floxuridine, shown below:

podophyllotoxin, shown below:



and related compounds can be included. The prodrug ester can be formed at the 2-hydroxy position for the "A" cis-platin derivative, the 2-hydroxyethyl position of the "B" cis-platin derivative, the 3' and 5' hydroxy positions of floxuridine and at the C-4 hydroxy for podophyllotoxin.

[0050] The parent compounds selected for prodrug forms need not be substantially water-insoluble, although the polymer-based prodrugs of the present invention are especially well suited for delivering such water-insoluble compounds. Other useful parent compounds include, for example, certain low molecular weight biologically active proteins, enzymes and peptides, including peptido glycans, as well as other anti-tumor agents, cardiovascular agents such as forskolin, anti-neoplastics such as combretastatin, vinblastine, vincristine, doxorubicin, AraC, maytansine, etc. anti-infectives such as vancomycin, erythromycin, etc. anti-fungals such as nystatin or amphoteracin B, anti-anxiety agents, gastrointestinal agents, central nervous system-activating agents, analgesics, fertility or contraceptive agents, anti-inflammatory agents, steroidal agents, anti-urecemic agents, cardiovascular agents, vasodilating agents, vasoconstricting agents and the like.

[0051] The foregoing is illustrative of the biologically active moieties which are suitable for the prodrugs of the present invention. It is to be understood that those biologically active materials not specifically mentioned but having suitable ester-forming groups, i.e. hydroxyl moieties, are also intended and are within the scope of the present invention. It is also to be understood that the prodrug conjugates of the present invention may also include compounds containing not only one equivalent of drug and polymer but also a moiety which does not effect bioactivity in vivo. For example, it has been found that in some instances, in spite of reacting diacids with drug molecules having a single linkage point,

the reaction conditions do not provide prodrugs with two equivalents of drug per polymer. On the contrary, the prodrugs contain only one equivalent of drug per polymer. By- products of the reactants such as acyl ureas can be formed. Furthermore, it has also been found that in spite of the reaction with a bis-activated polymer, the prodrugs are remarkably free of crosslinked species.

[0052] The only limitation on the types of molecules suitable for inclusion herein is that there is at least one position on which the hydrolyzable linkage can be attached, so that after prodrug administration, the prodrug can regenerate sufficient quantities of the parent compound in vivo.

E. SYNTHESIS OF PRODRUGS

[0053] Generally, the prodrugs of the invention are prepared by:

1) providing an activated polymer, such as a PEG-acid or PEG-diacid and a parent compound having a position thereon which will allow a hydrolyzable linkage to form, and
2) reacting the two substituents in an inert solvent such as methylene chloride, chloroform, toluene or DMF in the presence of a coupling reagent such as 1,3-diisopropylcarbodiimide (DIPC), 1,(3-dimethyl aminopropyl) 3-ethyl carbodiimide (EDC), any suitable dialkyl carbodiimide, Mukaiyama reagents, (e.g. 2-halo-1-alkyl-pyridinium halides) or propane phosphonic acid cyclic anhydride (PPACA), etc. which are available, for example from commercial sources such as Sigma Chemical, or synthesized using known techniques and a base such as dimethylaminopyridine (preferred), diisopropyl ethylamine, pyridine, triethylamine, etc. at a temperature from 0°C up to 22°C (room temperature).

[0054] In another preferred aspect of this embodiment, the synthesis method provides polymer-based prodrugs having a circulation half-life greater than their in-vivo hydrolysis half-life. The method includes:

reacting a biologically active moiety containing an available hydroxyl group with an amino acid spacer moiety containing an available carboxylic acid group in the presence of a first coupling agent to form a biologically active moiety - spacer prodrug intermediate,
reacting the biologically active moiety - spacer prodrug intermediate with a substantially non-antigenic polymer containing a terminal carboxylic acid group in the presence of a second coupling agent and recovering the polymer-based prodrug.

The first and second coupling agents can be the same or different.

[0055] Examples of suitable bifunctional spacer groups include *l*-alanine and *d*-alanine, etc.

[0056] An illustrative example of method of preparing the conjugates using camptothecin derivatives as the prototypical biologically active nucleophile includes the steps of:

forming a camptothecin derivative containing an amino acid spacer containing moiety, by contacting the camptothecin derivative with an amino acid spacer containing moiety such as tBoc-*d* or *l* alanine in the presence of a coupling agent such as DIPC or PPAC;
forming the trihaloacetic acid deriviative of the camptothecin derivative containing the amino acid spacer containing moiety such as the trifluoroacetic acid salt; and
reacting the trihaloacetic acid derivative of the camptothecin derivative containing the amino acid spacer containing moiety with a diacid derivative of a substantially non-antigenic polymer such as a PEG diacid. The resultant compound is recovered using known techniques.

[0057] Alternative and specific syntheses are provided in the examples. One particular alternative, however, includes derivatizing the biologically active moiety in the position desired for the linkage and thereafter reacting the derivative with an activated polymer.

G. METHODS OF TREATMENT

[0058] In the present invention various methods of treatment for various medical conditions in mammals are involved. The methods include administering to the mammal in need of such treatment, an effective amount of a prodrug, such as a paclitaxel 2'-PEG ester, which has been prepared as described herein. The compounds are useful for, among other things, treating neoplastic disease, reducing tumor burden, preventing metastasis of neoplasms and preventing recurrences of tumor/neoplastic growths in mammals.

[0059] The amount of the prodrug administered will depend upon the parent molecule included therein. Generally,

the amount of prodrug used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various prodrug compounds will vary somewhat depending upon the parent compound, rate of in vivo hydrolysis, molecular weight of the polymer, etc. In general, however, prodrug taxanes are administered in amounts ranging from about 5 to about 500 mg/m^2 per day, based on the amount of the taxane moiety. Camptothecin and podophyllotoxin prodrugs are also administered in amounts ranging from about 5 to about 500 mg/m^2 per day. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the prodrug selected based on clinical experience and the treatment indication.

[0060] The prodrugs of the present invention can be included in one or more suitable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral and/or parenteral routes depending upon the needs of the artisan. In preferred aspects of the invention, however, the prodrugs are parenterally administered to mammals in need thereof.

H. EXAMPLES

[0061] The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention. The numbers shown in bold in parentheses in the Examples correspond to the compounds shown in the schematic diagrams set forth in the Figures.

EXAMPLE 1 (intermediates)

a) Camptothecin-20-O-(*l*) Alanate TFA salt (23):

[0062] Referring now to Figure 1, tBoc-*l*-Alanine (1.8 g, 9.39 mmol) was dissolved in 700 mL of anhydrous methylene chloride at room temperature. To this solution, DIPC (1.5 ml 9.39 mmol), DMAP (765 mg, 6.26 mmol) and camptothecin (1.09 g, 3.13 mmol) were added at 0°C. The reaction mixture was allowed to warm to room temperature and left for 16 hours. The solution was washed with 0.1N HCl, dried and evaporated under reduced pressure to yield a white solid which was recrystallized from methanol to give Camptothecin-20-O-ester of t-Boc-*l*-Alanine 21. ^{1}H NMR(DMSO-D_6): δ 0.9(t), 1.3(d). 1.6(s), 2.1(m), 4(m), 5.3(s), 5.5(s), 7.3(s), 7.5-8.8(m).

[0063] Compound **21** (1.19 g, 2.12 mmol) was dissolved in a mixture of methylene chloride (15 ml) and trifluoroacetic acid (15 ml) and stirred at room temperature for I hour. The solvent was removed and the solid was recrystallized from methylene chloride and ether to give (I g) of product **23** as the TFA salt.

[0064] ^{1}H NMR(DMSO-D_6) δ: 1.0(t), 1.6(d), 2.2(m), 4.4(m), 5.4(s), 5.6(s), 7.2(s), 7.7-8.8(m).
^{13}C NMR (DMSO-D_6) δ: 7.5, 15.77, 30.09, 47.8, 50.27, 66.44, 77.5, 94.92, 119.10, 127.82, 128.03, 128.62, 128.84, 129.75, 130.55, 131.75, 144.27, 146.18, 147.90, 152.24, 156.45, 166.68, 168.69.

b) Camptothecin-20-O-(*d/l*) Alanate TFA salts:

[0065] The *d*-alanate and *d/l* racemic alanate were prepared using the same procedures outline above with the respective isomer replacing the tBoc-*l*-alanate used in Example 1 a).

EXAMPLE 2

Camptothecin-20-O-ester of PEG_{40kDa}L-Alanine - Compound 25: Method A:

I) PEG (40 kDa) dicarboxylic acid

a) Di-t-BUTYL ESTER OF PEG (40,000) DI-CARBOXYLIC ACID

[0066] A solution of 50 grams (1.3 mmoles) of PEG-$(OH)_2$ in 750 ml of toluene was azeotroped with the removal of 150 ml of distillate. The reaction mixture was then cooled to 30°C, followed by the addition of 4 ml (4.0 mmoles) of a 1.0 molar solution of potassium t-butoxide in t-butanol. The resulting mixture was stirred for 1 hour at room temperature, followed by the addition of 1.6 grams (8.0 mmoles) of t-butylbromoacetate. The resulting cloudy mixture was heated to reflux, followed by removal of the heat, and stirring for 18 hours at room temperature. The reaction mixture was filtered through celite and the solvent removed by rotary evaporator. The residue was recrystallized from methylene chloride/ethyl ether to yield 45.2 grams (86% yield). The named product, however was found to be over 99% pure, the starting material being present in an amount of less than 1.0%. ^{13}CNMR assignments: $(\underline{C}H_3)_3C$, 27.7 ppm; $(CH)_3\underline{C}$, 80.9 ppm; C=O, 169.1 ppm.

b) PEG (40,000) DI-CARBOXYLIC ACID

[0067] A solution of 20.0 grams (0.5 mmoles) of PEG (40,000) carboxylic acid t-butyl ester, 100 ml of trifluoroacetic acid, and 0.1 ml of water in 200 ml of methylene chloride was stirred at room temperature for 3 hours. The solvent was then removed by rotary evaporation, followed by recrystallization of the residue from methylene chloride/ethyl ether to yield 16.9 grams (84% yield) of product. Purity of the named product was confirmed to be in excess of 99%. ^{13}CNMR assignments: C=O, 170.9 ppm.

II Synthesis of the Camptothecin -20-O-Alanate PEG Derivative

[0068] Referring to Figure 1, PEG_{40kDa} diacid (6.5 g, 0.62 mmol) was dissolved in 60 mL of anhydrous methylene chloride at room temperature and to this solution at 0°C were added DIPC (148 µL, 0.97 mmol), DMAP (296 mg, 2.43 mmol) and compound 23 (627 mg, 0.97 mmol). The reaction mixture was allowed to warm to room temperature and left for 16 hours. The solution was washed with 0.1N HCl, dried and evaporated under reduced pressure to yield 25 as a white solid which was recrystallized from 2-propanol (5.5 g, 83.%). ^{13}C and ^{1}H NMR analysis confirmed the structure. ^{13}C NMR ($CDCl_3$) δ 6.81,16.93,30.80,
46.59,49.28,66.17,69.77,70.2-71(PEG),76.53,94.79,119.20,127.18,127.53,127.91,
128.95,129.72,130.68,144.58,145.76,148.05,151.46,156.37,165.99,168.87,170.32.

[0069] The procedure of II was repeated using propane phosphoric acid cyclic anhydride (PPACA) in place of DIPC in order to form the named compound.

[0070] The racemic mixture is prepared in the same manner.

III) Analysis of Camptothecin 20-O-ester of PEG_{40kDa} *l*-Alanine(25):

[0071] The UV absorbance of native camptothecin in methylene chloride was determined at 227 nm for five different concentrations ranging from 4 to 21 µM. From the standard plot of absorbance vs. concentration, the absorption coefficient for camptothecin was calculated to be 2.96×10^4 $Mol^{-1}Cm^{-1}$. Camptothecin compound 25 was dissolved in methylene chloride at an approximate concentration of 4 µM, and the UV absorbance of this compound at 227 nm was determined. Using this value, and employing the absorption coefficient obtained from above, the concentration of camptothecin in the sample was determined. Thus, dividing this value by the camptothecin-PEG ester concentration provided the percentage of camptothecin in the esters.

[0072] Determination of% of camptothecin in the product using the UV method indicated 2 eq. of camptothecin per PEG molecule.

EXAMPLE 3

Camptothecin-20-O-ester of PEG_{40kDa}(*d*/*l*)-Alanine - Compound 25: Method B:

[0073] Referring now to Figure 4 for guidance, compound **25** can also be prepared using a similar procedure to that shown below in Example 10 in order to prepare compound **31**, substituting PEG-*d*-alanine **28**, or PEG-*l*- alanine **29** (shown in Figure 2) in place of **27**.

EXAMPLE 4

Camptothecin-20-O-ester of PEG_{40kDa}(*d*)-Alanine - Compound 26: Method A:

[0074] As shown in Figure 1, the title compound is prepared in a similar manner as that used for preparing compound **25** in Example 2 using tBoc-*d*-Alanine as starting material.

EXAMPLE 5

Camptothecin-20-O-ester of PEG_{40kDa}(*d*)-Alanine - Compound 26: Method B:

[0075] Compound **26** is also prepared using a similar procedure to that described in Example 10 for preparing compound **31** and substituting PEG-*d*-Alanine **29** (See Figure 2) in place of **27**. The racemic alanine mixture can also be prepared using either of the foregoing procedures.

EXAMPLE 6 (not according to invention)

PEG_{40kDa}-β-Alanine(27):

[0076] As shown in Figure 2, PEG_{40kDa} diacid (3 g, 0.075 mmol) was dissolved in 30 mL of anhydrous methylene chloride at room temperature. To this solution at 0°C were added DIPC (91.4 µL 0.72 mmol), DMAP (128 mg, 1.04 mmol) and β-alanine-t-butylester (109 mg, 0.59 mmol). The reaction mixture was allowed to warm to room temperature after 3 hours and left for 16 hours. The solution was washed with 0.1N HCl, dried and evaporated under reduced pressure to yield PEG_{40kDa}β-alanine- t-butyl ester as a white solid which was dissolved in a mixture of methylene chloride (50 ml) and trifluoroacetic acid (25 ml) at 0°C for overnight. Solvent was removed and the solid was recrystallized from methylene chloride/ether to give 27 (2.3 g, 77%).
^{13}C NMR ($CDCl_3$) δ: 32.99, 33.62, 68.10, 69.72, 169.08, 172.04.

EXAMPLE 7 (intermediate)

PEG_{40kDa}-*d*-Alanine(28):

[0077] As shown in Figure 2, the title compound is prepared by using a similar procedure to that used for synthesizing compound **27** in Example 6, substituting (*d*)-alanine-t-butyl ester in place of β-alanine-t-butyl ester.

EXAMPLE 8 (intermediate)

PEG_{40kDa}-*l*-Alanine(29):

[0078] The title compound is prepared by using a similar procedure to that used for synthesizing compound **27** in Example 6, substituting (*l*)-alanine-t-butyl ester in place of β-alanine-t-butyl ester. (See Figure 2).

EXAMPLE 9

a) Paclitaxel-2'-O-ester of 27 - Compound 30a (not according to invention)

[0079] Referring to Figure 3, PEG_{40kDa} β-alanine (27, 2.3 g, 0.057 mmol) was dissolved in 20 mL of anhydrous methylene chloride at room temperature. To this solution at 0°C were added DIPC (32 µL, 0.2 mmol), DMAP (25 mg, 0.2 mmol) and paclitaxel (175.6 mg, 0.2 mmol). The reaction mixture was allowed to warm to room temperature and left for 16 hours. The solution was washed with 0.1N HCl, dried and evaporated under reduced pressure to yield **30a** as a white solid (2 g, 87%) which was recrystallized from 2-propanol.
[0080] ^{13}C NMR ($CDCl_3$) δ: 9.08, 14.22, 21.49, 21.89, 22.18, 25.9, 33.55, 34.90, 35.03, 35.21, 42.67, 46.9, 52.22, 57.51, 67.59-71.96 (PEG), 73.97, 74.60, 75.01, 80.11, 83.52, 126.32, 127.11, 127.57, 128.05, 128.17, 128.65, 129.50, 130.79, 131.96, 133.06, 136.75, 141.84, 165.97, 166.77, 167.45, 169.21, 169.70, 170.28, 170.33, 202.82.

b) Paclitaxel-2'-O-ester of 28 - Compound 30b (acc. to invention)

[0081] The procedure of Example 9a was repeated using *d*-alanine instead of β-alanine to yield compound **30b** (Figure 5).

c) Paclitaxel-2'-O-ester of 29 - Compound 30c (acc. to invention)

[0082] The procedure of Example 9a was repeated using *l*-alanine instead of β-alanine to yield compound **30c** (Figure 5).

EXAMPLE 10 (not according to invention)

Camptothecin 20-O-ester of 27 - Compound 31:

[0083] Referring now to Figure 4, PEG_{40kDa} β-alanine(27, 2.3 g, 0.057 mmol) is dissolved in 20 mL of anhydrous methylene chloride at room temperature and to this solution at 0°C are added DIPC (32 µL, 0.2 mmol), DMAP (25 mg, 0.2 mmol) and camptothecin (130 mg, 0.25 mmol). The reaction mixture is allowed to warm to room temperature and left for 16 hours. The solution is washed with 0·1N HCl, dried and evaporated under reduced pressure to yield **31**.

EXAMPLE 11 (intermediates)

a) PEG_{40kDa}phenylalanine (51):

[0084] PEG_{40kDa} diacid (9.5g, 0.23 mmol) was dissolved in 20 mL of anhyd methylene chloride at room temperature and to this solution at 0°C were added DIPC (141 µL, 0.92 mmol), DMAP (197 mg, 1.6 mmol) and phenylalanine-t-butyl ester (176.4mg, 0.92 mmol) at 0°C. The reaction mixture was allowed to warm to room temperature after 3 hours and left for 16 hours. The solution was washed with 0.1N HCl, dried and evaporated under reduced pressure to yield PEG_{40kDa}phenylalanine t-butyl ester as a white solid which was dissolved in a mixture of methylene chloride (50ml) and trifluoroaceticacid (25ml) at 0°C for overnight. Solvent was removed and the solid was recrystallized from methylene chloride/ether to give **51** (7.1g, 75%).
^{13}C NMR ($CDCl_3$) δ 39.42,69.59,70.19,169.39,169.46.

b) PEG_{40kDa}leucine (52):

[0085] PEG_{40kDa} diacid (9.5g, 0.23 mmol) was dissolved in 20 mL of anhyd. methylene chloride at room temperature and to this solution at 0°C were added DIPC (141 µL, 0.92 mmol), DMAP (197 mg, 1.6 mmol) and leucine-tbutyl ester (176.4mg, 0.92 mmol) at 0°C. The reaction mixture was allowed to warm to room temperature after 3 hours and left for 16 hours. The solution was washed with 0.1N HCl, dried and evaporated under reduced pressure to yield PEG_{40kDa}leucine t-butyl ester as a white solid which was dissolved in a mixture of methylene chloride (50ml)and trifluoroaceticacid (25ml) at 0°C for overnight. Solvent was removed and the solid was recrystallized from methylene chloride/ether to give **52** (7.1g, 75%). ^{13}C NMR ($CDCl_3$) δ 39.42,69.59,70.19,169.39,169.46.

c) PEG_{40kDa}Proline (53):

[0086] PEG_{40kDa} diacid (9.5g, 0.23 mmol) was dissolved in 20 mL of anhyd. methylene chloride at room temperature and to this solution at 0°C were added DIPC (141 µL, 0.92 mmol), DMAP (197 mg, 1.6 mmol) and proline-t-butylester (176.4mg, 0.92 mmol) at 0°C. The reaction mixture was allowed to warm to room temperature after 3 hours and left for 16 hours. The solution was washed with 0.1N HCl, dried and evaporated under reduced pressure to yield PEG_{40kDa}proline t-butyl ester as a white solid which was dissolved in a mixture of methylene chloride(50ml)and trifluoroaceticacid (25ml)at 0°C for overnight. Solvent was removed and the solid was recrystallized from methylene chloride/ether to give **53** (7.1g, 75%). ^{13}C NMR ($CDCl_3$) δ 39.42,69.59,70.19,169.39,169.46.

d) PEG_{40kDa}methionine (54):

[0087] PEG_{40kDa} diacid (9.5g, 0.23 mmol) was dissolved in 20 mL of anhyd. methylene chloride at room temperature and to this solution at 0°C were added DIPC (141 µL, 0.92 mmol), DMAP (197 mg, 1.6 mmol) and meththionine-t-butylester (176.4mg, 0.92 mmol) at 0°C. The reaction mixture was allowed to warm to room temperature after 3 hours and left for 16 hours. The solution was washed with 0. 1N HCl, dried and evaporated under reduced pressure to yield PEG_{40kDa} methionine t-butyl ester as a white solid which was dissolved in a mixture of methylene chloride (50ml) and trifluoroaceticacid (25ml)at 0°C for overnight. Solvent was removed and the solid was recrystallized from methylene chloride/ether to give **54** (7.1g, 75%). ^{13}C NMR ($CDCl_3$) δ 39.42,69.59,70.19,169.39,169.46.

EXAMPLE 12

Acyclovir-PEG prodrug:

[0088] PEG_{40kDa}L-alanine diacid (**29**, 11.5g, 0.287mmol) is dissolved in 200 mL of anhydrous methylene chloride at room temperature and to this solution at 0°C are added DIPC (0.175ml, 1.15mmol µL), DMAP (140 mg, 1.15mmol) and acyclovir (258 mg, 1.15 mmol). The reaction mixture is allowed to warm to room temperature after 2 hours and left for 16 hours. The solution is concentrated to about 100 ml and filtered through celite and the filterate is evaporated under reduced pressure to yield acyclovir-PEG prodrug as a solid which is recrystallized from CH_2Cl_2/ether.

EXAMPLE 13

Amoxicillin-PEG prodrug:

[0089] PEG_{40kDa}phenylalanine diacid (**51**, 11.5g, 0.287mmol) is dissolved in 200 mL of anhydrous methylene chloride

at room temperature and to this solution at 0°C are added DIPC (0.175ml, 1.15mmol μL), DMAP (140mg, 1.15mmol) and amoxicillin (419mg, 1.15 mmol). The reaction mixture is allowed to warm to room temperature after 2 hours and left for 16 hours. The solution is concentrated to about 100 ml and filtered through celite and the filterate is evaporated under reduced pressure to yield amoxicillin-PEG prodrug as a solid which is recrystallized from CH_2Cl_2/ether.

EXAMPLE 14

Fluconazole-PEG prodrug:

[0090] PEG_{40kDa}leucine diacid (**52**, 11.5g, 0.287mmol) is dissolved in 200 mL of anhydrous methylene chloride at room temperature and to this solution are added DIPC (0.175ml, 1.15mmol μL), DMAP (140mg, 1.15mmol) and fluconazole (352mg, 1.15 mmol) at 0°C. The reaction mixture is allowed to warm to room temperature after 2 hours and left for 16 hours. The solution is concentrated to about 100 ml and filtered through celite and the filterate is evaporated under reduced pressure to yield fluconazole-PEG prodrug as a solid which is recrystallized from CH_2Cl_2/ether.

EXAMPLE 15

Floxuridine-PEG prodrug:

[0091] PEG_{40kDa}proline diacid (53, 0.5g, 0.0125mmol,) is dissolved in 20 mL of anhydrous methylene chloride at room temperature and to this solution are added 2-chloro-1-methylpyridinium iodide (17mg, 0.067 mmol), DMAP (17mg, 0.14mmol) and floxuridine (13mg, 0.049mmol) at 0°C. The reaction mixture is allowed to warm to room temperature after 2 hours and left for 16 hours. The solution is concentrated to about 100 ml and filtered through celite and the filterate is evaporated under reduced pressure to yield floxuridine-PEG prodrug as a solid which is recrystallized from CH_2Cl_2/ether.

Claims

1. A prodrug compound having the formula (I) or (II):

$$D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}R^2 \quad (I)$$

$$[D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}]_2R^3 \quad (II)$$

wherein
D is a residue of a drug having an ester-forming hydroxyl group,
R^2 and R^3 are a residue of a water soluble polyalkylene oxide or activated polyalkylene oxide having a molecular weight of from 20,000 to 80,000, and
the moiety -CO-CH(R^1)-NH- is a residue of an (*l*) amino acid, a (*d*) amino acid or a mixture of (*l*) and (*d*) amino acid.

2. A prodrug compound according to claim 1, wherein said amino acid is selected from the group consisting of (*d*) and/or (*l*) alanine and phenylalanine.

3. A prodrug compound according to claim 1 or 2, wherein said polyalkylene oxide is a polyethylene glycol.

4. A prodrug compound according to any one of claims 1-3, wherein said polyalkylene oxide has a molecular weight of from 25,000 to 45,000.

5. A prodrug compound according to claim 4, wherein said polyalkylene oxide has a molecular weight of from 30,000 to 42,000.

6. A prodrug compound according to any one of claims 1-5, wherein R^2 is capped with a C_{1-4} alkyl group.

7. A prodrug compound according to any one of claims 1-6, wherein said drug is selected from the group consisting

of biologically active proteins, enzymes, peptides, anti-tumor agents, cardiovascular agents, anti-neoplastics, anti-infectives, anti-fungals, anti-anxiety agents, gastrointestinal agents, central nervous system-activating agents, analgesics, fertility agents, contraceptive agents, anti-inflammatory agents, steroidal agents, anti-urecemic agents, vasodilating agents, and vasoconstricting agents.

8. A prodrug compound according to claim 7, wherein said drug is selected from the group consisting of paclitaxel, taxotere, camptothecin, podophyllotoxin, and floxuridine.

9. A prodrug compound according to claim 1, having the formula

$$D\text{-}O\text{-}CO\text{-}CH(CH_3)\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH(CH_3)\text{-}CO\text{-}O\text{-}D$$

wherein D is a residue of the drug camptothecin bound via the 20(S)-OH group, the moiety $-CO-CH(CH_3)-NH-$ is a residue of (*l*) or (*d*) alanine, and PEG is polyethylene glycol with a molecular weight of about 40 kDa.

10. A prodrug compound according to claim 1, having the formula

$$D\text{-}O\text{-}CO\text{-}CH(CH_3)\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH(CH_3)\text{-}CO\text{-}O\text{-}D$$

wherein D is a residue of the drug paclitaxel bound via the 2'-OH group, the moiety $-CO-CH(CH_3)-NH-$ is a residue of (*l*) or (*d*) alanine, and PEG is polyethylene glycol with a molecular weight of about 40 kDa.

11. A prodrug compound according to claim 1, having the formula

$$D\text{-}O\text{-}CO\text{-}CH(CH_3)\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH(CH_3)\text{-}CO\text{-}O\text{-}D$$

wherein D is a residue of the drug acyclovir, the moiety $-CO-CH(CH_3)-NH-$ is a residue of (*l*) alanine, and PEG is polyethylene glycol with a molecular weight of about 40 kDa.

12. A prodrug compound according to claim 1, having the formula

$$D\text{-}O\text{-}CO\text{-}CH(CH_2C_6H_5)\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH(CH_2C_6H_5)\text{-}CO\text{-}O\text{-}D$$

wherein D is a residue of the drug amoxicillin, the moiety $-CO-CH(CH_2C_6H_5)-NH-$ is a residue of phenylalanine, and PEG is polyethylene glycol with a molecular weight of about 40 kDa.

13. A prodrug compound according to claim 1, having the formula

$$D\text{-}O\text{-}CO\text{-}CH[CH_2CH(CH_3)_2]\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH[CH_2CH(CH_3)_2]\text{-}CO\text{-}O\text{-}D$$

wherein D is a residue of the drug fluconazole, the moiety $-CO-CH[CH_2CH(CH_3)_2]-NH-$ is a residue of leucine, and PEG is polyethylene glycol with a molecular weight of about 40 kDa.

14. A prodrug compound according to claim 1, having the formula

$$D\text{-}O\text{-proline-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-proline-}O\text{-}D$$

wherein D is a residue of the drug floxuridine, the moiety proline is a residue of proline, and PEG is polyethylene glycol with a molecular weight of about 40 kDa.

15. A prodrug compound according to any one of the preceding claims for use as a medicament.

16. Use of a prodrug compound according to any one of claims 1-14 for the manufacture of a medicament.

17. Use according to claim 16 wherein the (*l*) and/or (*d*) amino acid linkage between the drug moiety and polyalkylene oxide moiety is chosen such as to achieve a desirable balance between the rate of linkage hydrolysis and the rate of prodrug clearance from the body.

18. A method of preparing a prodrug compound as defined in any one of the claims 1-14 having a circulation half-life greater than its in-vivo hydrolysis half-life, comprising:

reacting a drug moiety having an ester-forming hydroxyl group with an amino acid spacer moiety containing an available carboxylic acid group in the presence of a first coupling agent to form a drug - spacer prodrug intermediate,
reacting said drug - spacer prodrug intermediate with a water soluble polyalkylene oxide or activated polyalkylene oxide having a molecular weight of from 20,000 to 80,000 and containing a terminal carboxylic acid group in the presence of a second coupling agent and recovering the prodrug compound.

19. A method according to claim 18, wherein said drug is selected from the group consisting of biologically active proteins, enzymes, peptides, anti-tumor agents, cardiovascular agents, anti-neoplastics, anti-infectives, anti-fungals, anti-anxiety agents, gastrointestinal agents, central nervous system-activating agents, analgesics, fertility agents, contraceptive agents, anti-inflammatory agents, steroidal agents, anti-urecemic agents, vasodilating agents, and vasoconstricting agents.

20. A method according to claim 18 or 19, wherein said amino acid spacer moiety is selected from the group consisting of (*l*) amino acids, (*d*) amino acids and a mixture of (*l*) and (*d*) amino acids.

21. A method according to claim 20, wherein said amino acid spacer moiety is selected from the group consisting of (*l*) and/or (*d*) alanine and phenylalanine.

22. A method according to any one of claims 18-21, wherein said first and said second coupling agents are independently selected from the group consisting of 1,3-diisopropylcarbodiimide (DIPC), dialkyl carbodiimides, 2-halo-1-alkyl-pyridinium halides, 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic anhydride (PPACA) and carbodiimide (CDI).

23. A method according to any one of claims 18-22, wherein said polyalkylene oxide is a polyethylene glycol.

Patentansprüche

1. Prodrug-Verbindung der Formel (I) oder (II)

$$D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}R^2 \quad (I)$$

$$[D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}]_2R^3 \quad (II)$$

worin
D der Rest eines Arzneimittels mit einer esterbildenden Hydroxygruppe ist,
R^2 und R^3 der Rest eines wasserlöslichen Polyalkylenoxids oder aktivierten Polyalkylenoxids mit einem Molekulargewicht von 20.000 bis 80.000 sind und
der Anteil -CO-CH(R^1)-NH- der Rest einer (*ℓ*)-Aminosäure, einer (d)-Aminosäure oder einer Mischung einer (*ℓ*)- und einer (d)-Aminosäure ist.

2. Prodrug-Verbindung gemäss Anspruch 1, worin die Aminosäure ausgewählt ist aus der Gruppe, bestehend aus (d)- und/oder (*ℓ*)-Alanin und Phenylalanin.

3. Prodrug-Verbindung gemäss Anspruch 1 oder 2, worin das Polyalkylenoxid ein Polyethylenglykol ist.

4. Prodrug-Verbindung gemäss einem der Ansprüche 1 bis 3, worin das Polyalkylenoxid ein Molekulargewicht von 25.000 bis 45.000 besitzt.

5. Prodrug-Verbindung gemäss Anspruch 4, worin das Polyalkylenoxid ein Molekulargewicht von 30.000 bis 42.000 besitzt.

6. Prodrug-Verbindung gemäss einem der Ansprüche 1 bis 5, worin R^2 mit einer C_{1-4}-Alkylgruppe verkappt ist.

7. Prodrug-Verbindung gemäss einem der Ansprüche 1 bis 6, worin das Arzneimittel ausgewählt ist aus der Gruppe, bestehend aus biologisch aktiven Proteinen, Enzymen, Peptiden, Antitumormitteln, kardiovaskulären Mitteln, Antineoplastika, infektionsverhindernden Mitteln, Antimykotika, Anxiolytika, gastrointestinalen Mitteln, das Zentralnervensystem aktivierenden Mitteln, Analgetika, fertilitätssteigernden Mitteln, Kontrazeptiva, entzündungshemmenden Mitteln, Steroiden, antiurämischen Mitteln, Vasodilatatoren und Vasokonstriktoren.

8. Prodrug-Verbindung gemäss Anspruch 7, worin das Arzneimittel ausgewählt ist aus der Gruppe, bestehend aus Paclitaxel, Taxoter, Camptothecin, Podophyllotoxin und Floxuridin.

9. Prodrug-Verbindung gemäss Anspruch 1 mit der Formel

$$\text{D-O-CO-CH(CH}_3\text{)-NH-CO-CH}_2\text{-O-PEG-O-CH}_2\text{-}$$

$$\text{CO-NH-CH(CH}_3\text{)-CO-O-D}$$

worin D ein Rest des über die 20(S)-OH-Gruppe gebundenen Arzneimittels Camptothecin ist, der Anteil -CO-CH (CH_3)-NH- ein Rest von (ℓ)- oder (d)-Alanin ist und PEG Polyethylenglykol mit einem Molekulargewicht von etwa 40 kDa ist.

10. Prodrug-Verbindung gemäss Anspruch 1 mit der Formel

$$\text{D-O-CO-CH(CH}_3\text{)-NH-CO-CH}_2\text{-O-PEG-O-CH}_2\text{-}$$

$$\text{CO-NH-CH(CH}_3\text{)-CO-O-D}$$

worin D der Rest des über die 2'-OH-Gruppe gebundenen Arzneimittels Paclitaxel ist, der Anteil -CO-CH(CH_3) -NH- ein Rest von (ℓ)- oder (d)-Alanin ist und PEG Polyethylenglykol mit einem Molekulargewicht von etwa 40 kDa ist.

11. Prodrug-Verbindung gemäss Anspruch 1 mit der Formel

$$\text{D-O-CO-CH(CH}_3\text{)-NH-CO-CH}_2\text{-O-PEG-O-CH}_2\text{-}$$

$$\text{CO-NH-CH(CH}_3\text{)-CO-O-D}$$

worin D der Rest des Arzneimittels Acyclovir ist, der Anteil -CO-CH(CH_3)-NH- ein Rest von (ℓ)-Alanin ist und PEG Polyethylenglykol mit einem Molekulargewicht von etwa 40 kDa ist.

12. Prodrug-Verbindung gemäss Anspruch 1 mit der Formel

$$\text{D-O-CO-CH(CH}_2\text{C}_6\text{H}_5\text{)-NH-CO-CH}_2\text{-O-PEG-O-CH}_2\text{-CO-}$$

$$\text{NH-CH(CH}_2\text{C}_6\text{H}_5\text{)-CO-O-D}$$

worin D der Rest des Arzneimittels Amoxicillin ist, der Anteil -CO-CH$(CH_2C_6H_5)$-NH- ein Rest von Phenylalanin ist und PEG Polyethylenglykol mit einem Molekulargewicht von etwa 40 kDa ist.

13. Prodrug-Verbindung gemäss Anspruch 1 mit der Formel

$$D\text{-}O\text{-}CO\text{-}CH[CH_2CH(CH_3)_2]\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}$$

$$CH_2\text{-}CO\text{-}NH\text{-}CH[CH_2CH(CH_3)_2]\text{-}CO\text{-}O\text{-}D$$

worin D ein Rest des Arzneimittels Fluconazol ist, der Anteil $-CO-CH[CH_2CH(CH_3)_2]-NH-$ ein Rest von Leucin ist und PEG Polyethylenglykol mit einem Molekulargewicht von etwa 40 kDa ist.

14. Prodrug-Verbindung gemäss Anspruch 1 mit der Formel

$$D\text{-}O\text{-}Prolin\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-}Prolin\text{-}O\text{-}D$$

worin D ein Rest des Arzneimittels Floxuridin ist, der Anteil Prolin ein Rest von Prolin ist und PEG Polyethylenglykol mit einem Molekulargewicht von etwa 40 kDa ist.

15. Prodrug-Verbindung gemäss einem der vorangehenden Ansprüche zur Verwendung als Medikament.

16. Verwendung einer Prodrug-Verbindung gemäss einem der Ansprüche 1 bis 14 zur Herstellung eines Medikaments.

17. Verwendung gemäss Anspruch 16, worin die (ℓ)- und/oder (d)-Aminosäureverknüpfung zwischen dem Arzneimittelanteil und dem Polyalkylenoxidanteil derart ausgewählt ist, dass ein gewünschtes Gleichgewicht zwischen der Verknüpfungshydrolyserate und der Prodrug-Clearancerate aus dem Körper erzielt wird.

18. Verfahren zur Herstellung einer Prodrug-Verbindung gemäss einem der Ansprüche 1 bis 14, mit einer Zirkulationshalbwertszeit, die höher als ihre in vivo-Hydrolysehalbwertszeit ist, umfassend:

Umsetzen eines Arzneimittelanteils mit einer esterbildenden Hydroxygruppe, mit einem Aminosäurespaceranteil, enthaltend eine verfügbare Carbonsäuregruppe, in Gegenwart eines ersten Kupplungsmittels zur Bildung eines Arzneimittelspacer-Prodrug-Zwischenprodukts,

Umsetzen des Arzneimittelspacer-Prodrug-Zwischenprodukts mit einem wasserlöslichen Polyalkylenoxid oder aktivierten Polyalkylenoxid mit einem Molekulargewicht von 20.000 bis 80.000 und enthaltend eine terminale Carbonsäuregruppe in Gegenwart eines zweiten Kupplungsmittels, und Rückgewinnung der Prodrug-Verbindung.

19. Verfahren gemäss Anspruch 18, worin das Arzneimittel ausgewählt ist aus der Gruppe, bestehend aus biologisch aktiven Proteinen, Enzymen, Peptiden, Antitumormitteln, kardiovaskulären Mitteln, Antineoplastika, infektionsverhindernden Mitteln, Antimykotika, Anxiolytika, gastrointestinalen Mitteln, das Zentralnervensystem aktivierenden Mitteln, Analgetika, fertilitätssteigernden Mitteln, Kontrazeptiva, entzündungshemmenden Mitteln, Steroiden, antiurämischen Mitteln, Vasodilatatoren und Vasokonstriktoren.

20. Verfahren gemäss Anspruch 18 oder 19, worin der Aminosäurespaceranteil ausgewählt ist aus der Gruppe, bestehend aus (ℓ) -Aminosäuren, (d)-Aminosäuren und einer Mischung aus (ℓ)- und (d)-Aminosäuren.

21. Verfahren gemäss Anspruch 20, worin der Aminosäurespaceranteil ausgewählt ist aus der Gruppe, bestehend aus (ℓ)- und/oder (d)-Alanin und Phenylalanin.

22. Verfahren gemäss einem der Ansprüche 18 bis 21, worin das erste und das zweite Kupplungsmittel unabhängig ausgewählt sind aus der Gruppe, bestehend aus 1,3-Diisopropylcarbodiimid (DIPC), Dialkylcarbodiimiden, 2-Halogen-1-alkylpyridiniumhalogeniden, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimiden (EDC), Propanphosphonsäurecycloanhydrid (PPACA) und Carbodiimid (CDI).

23. Verfahren gemäss einem der Ansprüche 18 bis 22, worin das Polyalkylenoxid ein Polyethylenglykol ist.

Revendications

1. Composé précurseur de médicament ayant pour formule (I) ou, (II) :

$$D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}R^2 \quad (I)$$

$$[D\text{-}O\text{-}CO\text{-}CH(R^1)\text{-}NH\text{-}]_2R^3 \quad (II)$$

où
D est un reste provenant d'un médicament ayant un groupe hydroxyle formant un ester,
R^2 et R^3 sont un reste d'un oxyde de polyalkylène ou d'un oxyde de polyalkylène activé soluble dans l'eau ayant une masse moléculaire de 20 000 à 80 000, et
le fragment -CO-CH(R^1)-NH- est un reste d'un (*l*) aminoacide ou d'un (*d*) aminoacide ou d'un mélange de (*l*) et (*d*) aminoacide.

2. Composé précurseur de médicament selon la revendication 1, dans lequel ledit aminoacide est choisi dans le groupe constitué par la (*d*) et/ou la (*l*) alanine et la phénylalanine.

3. Composé précurseur de médicament selon la revendication 1 ou 2, dans lequel ledit oxyde de polyalkylène est un polyéthylèneglycol.

4. Composé précurseur de médicament selon l'une quelconque des revendications 1 à 3, dans lequel ledit oxyde de polyalkylène a une masse moléculaire de 25 000 à 45 000.

5. Composé précurseur de médicament selon la revendication 4, dans lequel ledit oxyde de polyalkylène a une masse moléculaire de 30 000 à 42 000.

6. Composé précurseur de médicament selon l'une quelconque des revendications 1 à 5, dans lequel R^2 est coiffé par un groupe alkyle en C_{1-4}.

7. Composé précurseur de médicament selon l'une quelconque des revendications 1 à 6, ledit médicament étant choisi dans le groupe constitué par les protéines biologiquement actives, les enzymes, les peptides, les agents anti-tumoraux, les agents cardiovasculaires, les antinéoplasiques, les antiinfectieux, les antifongiques, les agents anti-anxiété, les agents gastro-intestinaux, les agents activant le système nerveux central, les analgésiques, les agents de la fertilité, les agents contraceptifs, les agents anti-inflammatoires, les agents stéroïdiens, les agents anti-uricémiques, les agents vasodilatateurs et les agents vasoconstricteurs.

8. Composé précurseur de médicament selon la revendication 7, ledit médicament étant choisi dans le groupe constitué par le paclitaxel, le taxotère, la camptothécine, la podophyllotoxine et la floxuridine.

9. Composé précurseur de médicament selon la revendication 1, ayant pour formule

$$D\text{-}O\text{-}CO\text{-}CH(CH_3)\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH(CH_3)\text{-}CO\text{-}O\text{-}D$$

où D est un reste du médicament camptothécine lié par l'intermédiaire du groupe 20(S)-OH, le fragment -CO-CH(CH_3)-NH- est un reste de (*l*) ou de (*d*) alanine et PEG est un polyéthylèneglycol ayant une masse moléculaire d'environ 40 kDa.

10. Composé précurseur de médicament selon la revendication 1, ayant pour formule

$$D\text{-}O\text{-}CO\text{-}CH(CH_3)\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}PEG\text{-}O\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH(CH_3)\text{-}CO\text{-}O\text{-}D$$

où D est un reste du médicament paclitaxel lié par l'intermédiaire du groupe 2'-OH, le fragment -CO-CH(CH_3)-NH-

est un reste de (*l*) ou de (*d*) alanine et PEG est un polyéthylèneglycol ayant une masse moléculaire d'environ 40 kDa.

11. Composé précurseur de médicament selon la revendication 1, ayant pour formule

où D est un reste du médicament acyclovir, le fragment -CO-CH(CH_3)-NH- est un reste de (*l*) alanine et PEG est un polyéthylèneglycol ayant une masse moléculaire d'environ 40 kDa.

12. Composé précurseur de médicament selon la revendication 1, ayant pour formule

où D est un reste du médicament amoxicilline, le fragment -CO-CH($CH_2C_6H_5$)-NH- est un reste de phénylalanine et PEG est un polyéthylèneglycol ayant une masse moléculaire d'environ 40 kDa.

13. Composé précurseur de médicament selon la revendication 1, ayant pour formule

où D est un reste du médicament fluconazole, le fragment -CO-CH[$CH_2CH(CH_3)_2$]-NH- est un reste de leucine et PEG est un polyéthylèneglycol ayant une masse moléculaire d'environ 40 kDa.

14. Composé précurseur de médicament selon la revendication 1, ayant pour formule

où D est un reste du médicament floxuridine, le fragment proline est un reste de proline et PEG est un polyéthylèneglycol ayant une masse moléculaire d'environ 40 kDa.

15. Composé précurseur de médicament selon l'une quelconque des revendications précédentes pour son utilisation en tant que médicament.

16. Utilisation d'un composé précurseur de médicament selon l'une quelconque des revendications 1 à 14 pour la fabrication d'un médicament.

17. Utilisation selon la revendication 16, où le lien (*l*) et/ou (*d*) aminoacide entre le fragment médicament et le fragment oxyde de polyalkylène est choisi de telle façon que l'équilibre souhaitable entre la vitesse d'hydrolyse du lien et la vitesse de la clairance du précurseur de médicament de l'organisme soit atteint.

18. Méthode de préparation d'un composé précurseur de médicament selon l'une quelconque des revendications 1 à 14 ayant une demi-vie dans la circulation supérieure à la demi-vie d'hydrolyse *in vivo*, comprenant :

la réaction d'un fragment de médicament ayant un groupe hydroxyle formant un ester avec un aminoacide contenant un groupe acide carboxyle disponible, fragment d'espacement, en présence d'un premier agent de couplage, pour former un intermédiaire de précurseur de médicament, médicament - fragment d'espacement, la réaction dudit intermédiaire de précurseur de médicament, médicament - fragment d'espacement, avec un oxyde de polyalkylène ou un oxyde de polyalkylène activé soluble dans l'eau, ayant une masse moléculaire de 20 000 à 80 000 et contenant un groupe acide carboxylique terminal, en présence d'un second agent de couplage et la récupération du composé précurseur de médicament.

19. Méthode selon la revendication 18, ledit médicament étant choisi dans le groupe constitué par les protéines biologiquement actives, les enzymes, les peptides, les agents anti-tumoraux, les agents cardiovasculaires, les anti-

néoplasiques, les antiinfectieux, les antifongiques, les agents anti-anxiété, les agents gastro-intestinaux, les agents activant le système nerveux central, les analgésiques, les agents de la fertilité, les agents contraceptifs, les agents anti-inflammatoires, les agents stéroïdiens, les agents anti-uricémiques, les agents vasodilatateurs et les agents vasoconstricteurs.

20. Méthode selon la revendication 18 ou 19, ledit aminoacide, fragment d'espacement, étant choisi dans le groupe constitué par les (*l*) aminoacides, les (*d*) aminoacides et un mélange de (*l*) et (*d*) aminoacides.

21. Méthode selon la revendication 20, ledit aminoacide, fragment d'espacement, étant choisi dans le groupe constitué par la (*l*) et/ou la (*d*) alanine et la phénylalanine.

22. Méthode selon l'une quelconque des revendications 18 à 21, où ledit premier agent de couplage et ledit second agent de couplage sont choisis, de façon indépendante, dans le groupe constitué par le 1,3-diisopropylcarbodiimide (DIPC), les dialkylcarbodiimides, les halogénures de 2-halogéno-1-alkylpyridinium, le 1-(3-diméthylaminopropyl)-3-éthylcarbodiimide (EDC), l'anhydride cyclique de l'acide propanephosphonique (PPACA) et le carbodiimide (CDI).

23. Méthode selon l'une quelconque des revendications 18 à 22, où ledit oxyde de polyalkylène est un polyéthylèneglycol.

FIG - 1

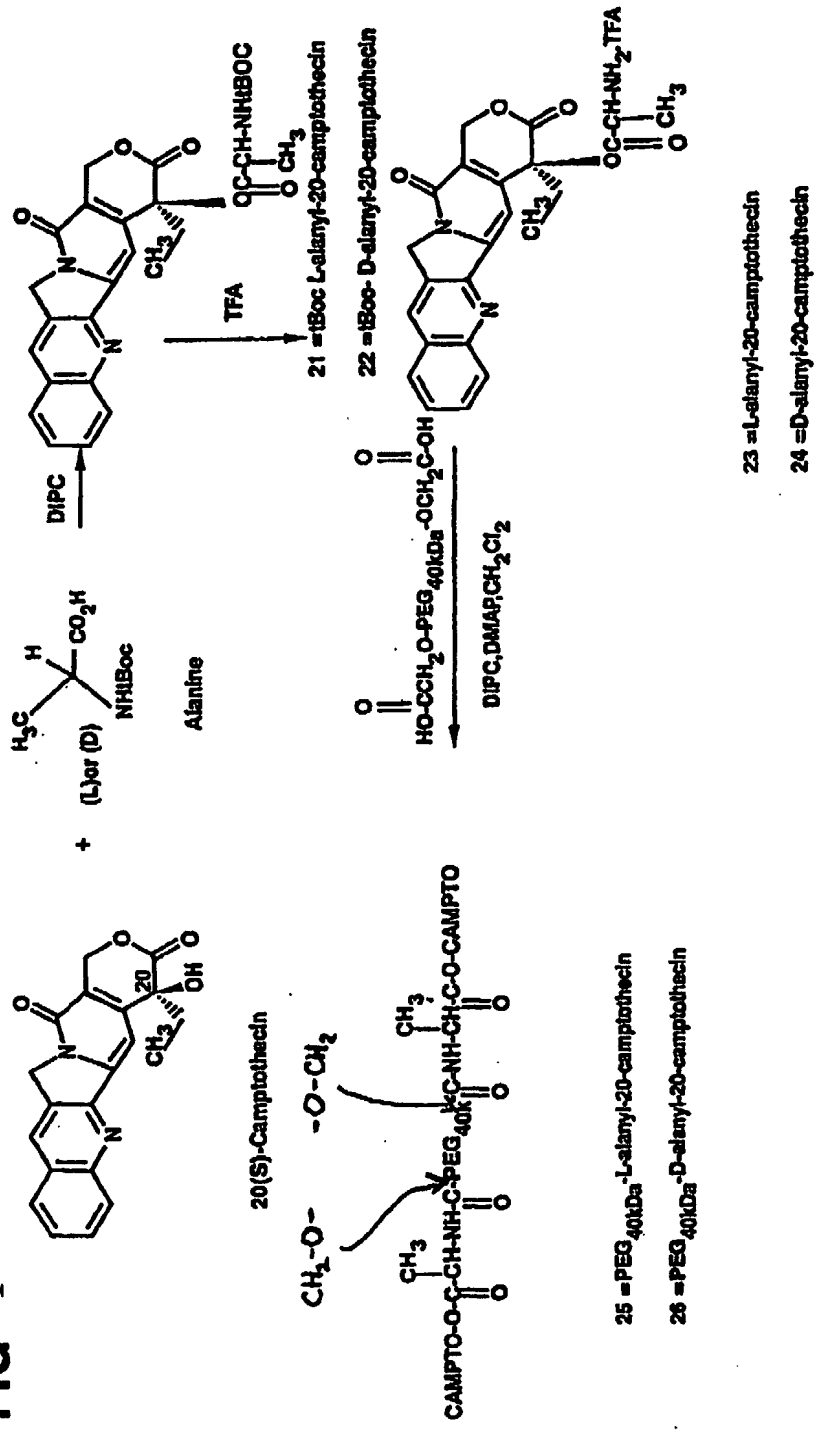
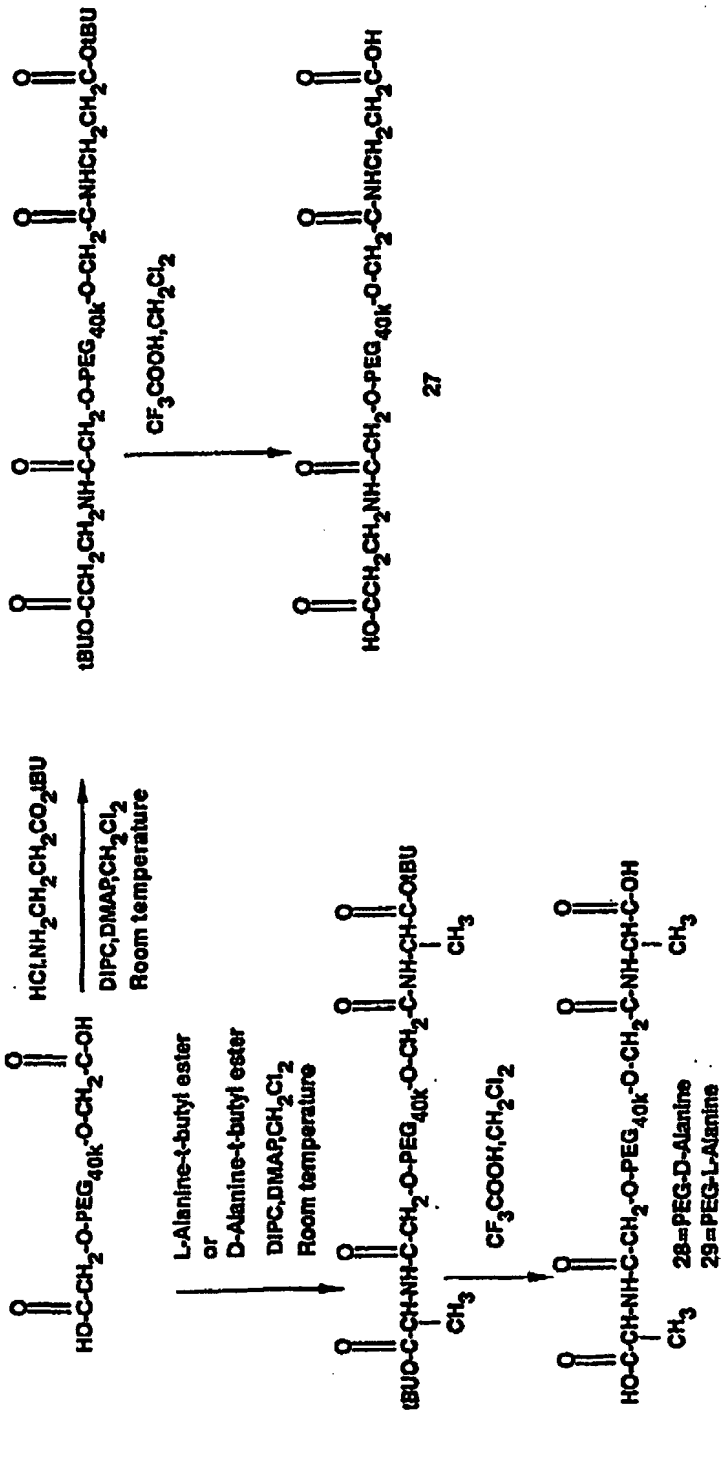

20(S)-Camptothecin
Alanine
(L)or (D)
DIPC
TFA
21 =tBoc L-alanyl-20-camptothecin
22 =tBoc- D-alanyl-20-camptothecin
DIPC,DMAP,CH2Cl2
23 =L-alanyl-20-camptothecin
24 =D-alanyl-20-camptothecin
25 =PEG40kDa-L-alanyl-20-camptothecin
26 =PEG40kDa-D-alanyl-20-camptothecin

FIG - 2


HO-C-CH2-O-PEG40k-O-CH2-C-OH
HCl.NH2CH2CH2CO2tBU
DIPC,DMAP,CH2Cl2
Room temperature
tBUO-CCH2CH2NH-C-CH2-O-PEG40k-O-CH2-C-NHCH2CH2C-OtBU
CF3COOH,CH2Cl2
HO-CCH2CH2NH-C-CH2-O-PEG40k-O-CH2-C-NHCH2CH2C-OH
27
L-Alanine-t-butyl ester
or
D-Alanine-t-butyl ester
DIPC,DMAP,CH2Cl2
Room temperature
tBUO-C-CH-NH-C-CH2-O-PEG40k-O-CH2-C-NH-CH-C-OtBU
CH3
CF3COOH,CH2Cl2
HO-C-CH-NH-C-CH2-O-PEG40k-O-CH2-C-NH-CH-C-OH
CH3
28=PEG-D-Alanine
29=PEG-L-Alanine

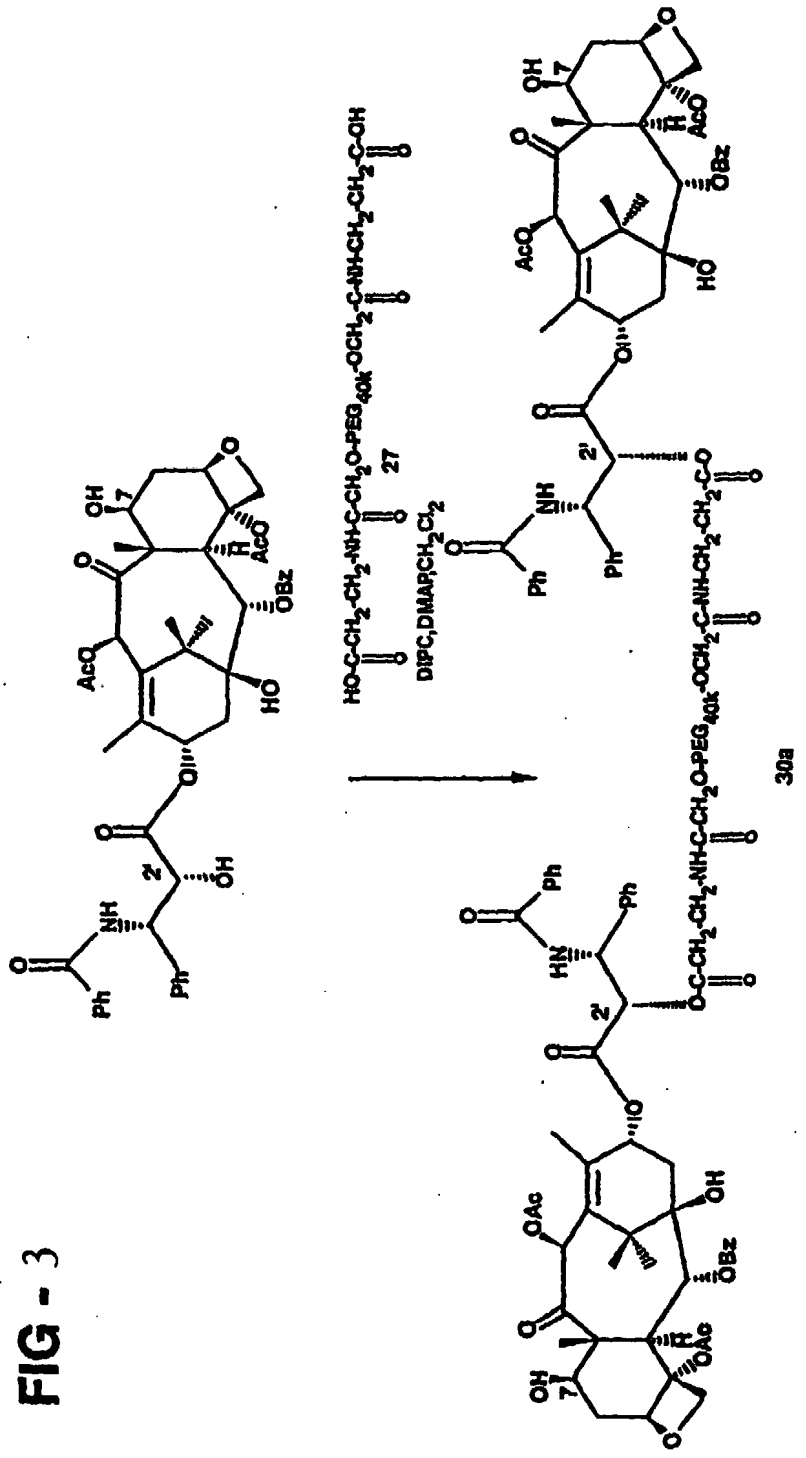

HO-C-CH2-CH2-NH-C-CH2O-PEG40k-OCH2-C-NH-CH2-CH2-C-OH
27
DIPC,DMAP,CH2Cl2
30a

FIG - 3

FIG - 4

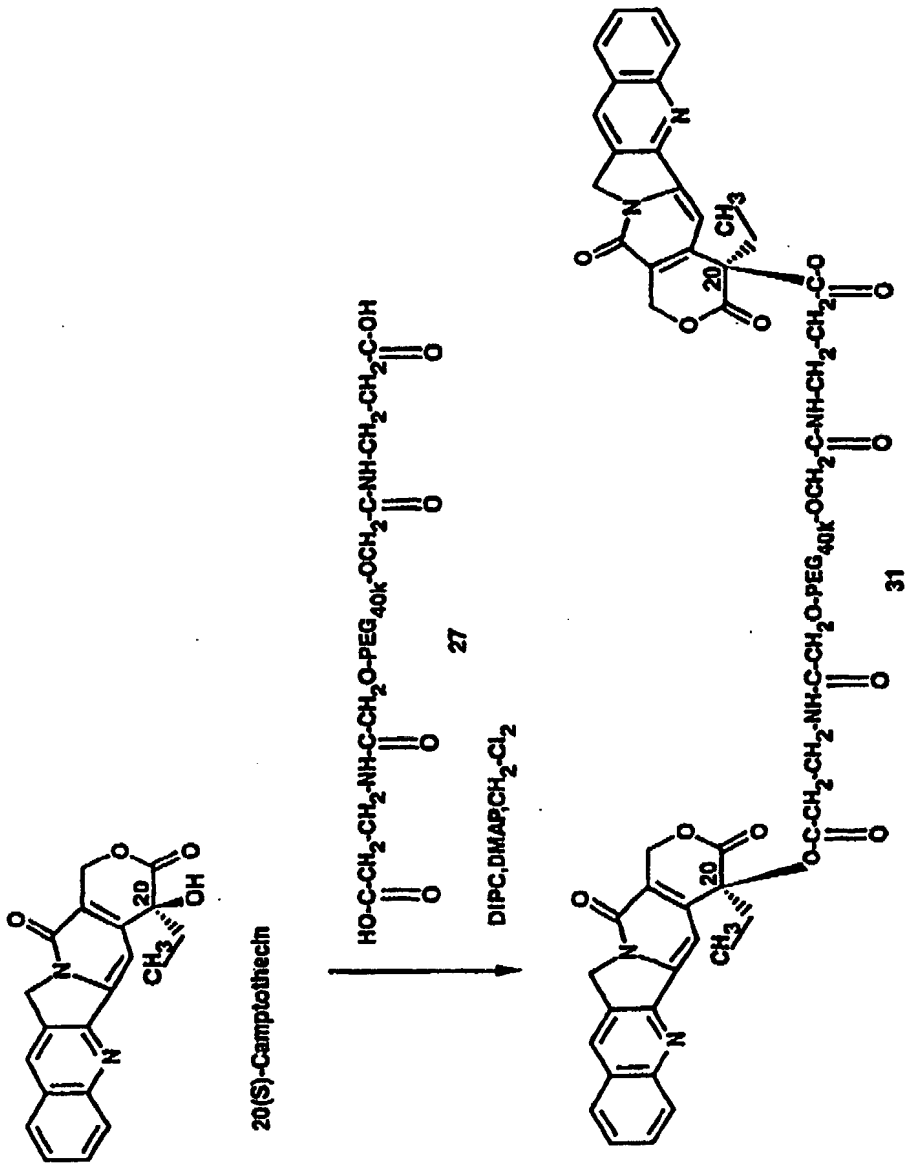
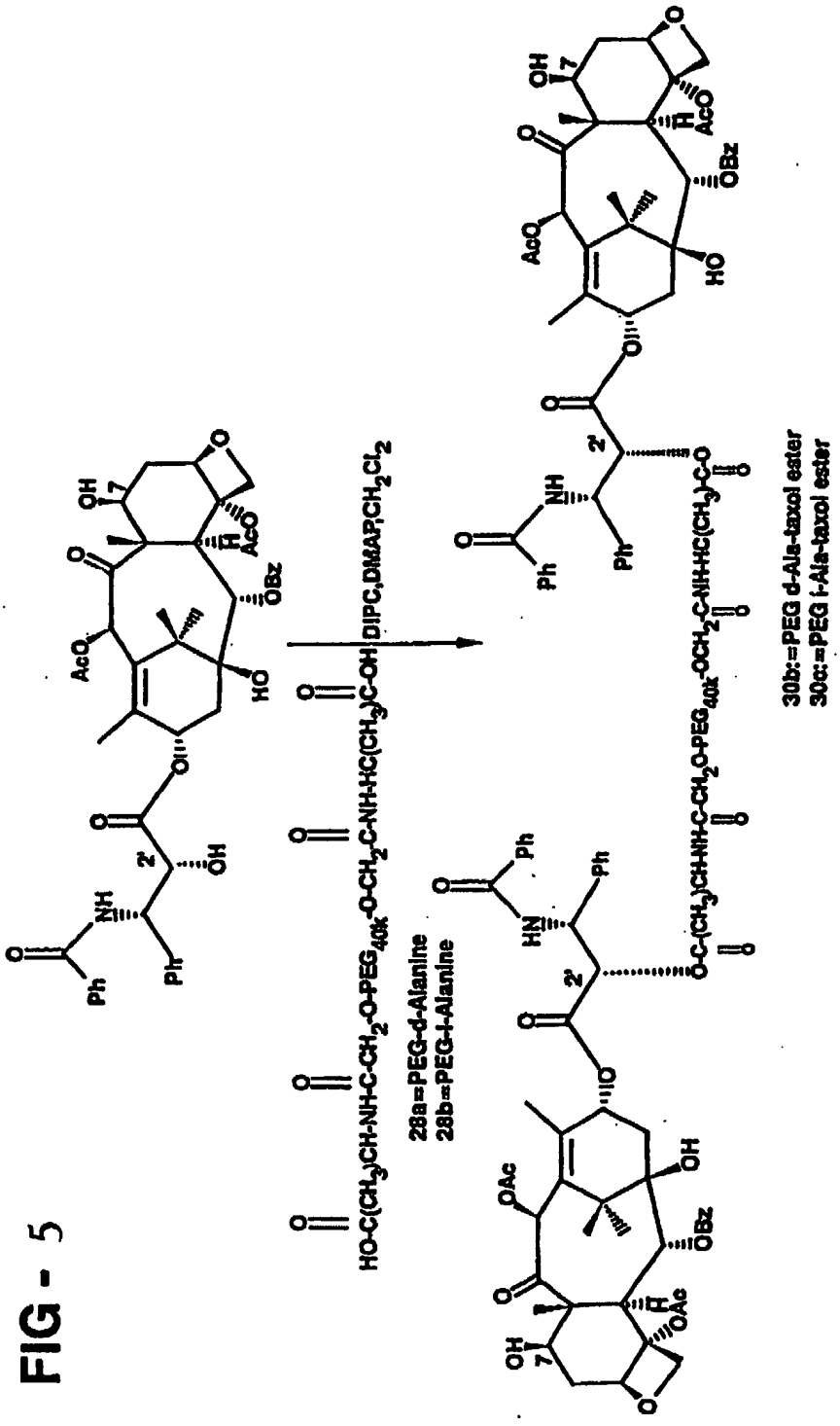

20(S)-Camptothecin
HO-C-CH2-CH2-NH-C-CH2O-PEG40k-OCH2-C-NH-CH2-CH2-C-OH
27
DIPC,DMAP,CH2-Cl2
O-C-CH2-CH2-NH-C-CH2O-PEG40k-OCH2-C-NH-CH2-CH2-C-O
31

FIG - 5


HO-C(CH3)CH-NH-C-NH-CH2-O-PEG40k-O-CH2-C-NH-HC(CH3)C-OH
DIPC,DMAP,CH2Cl2
28a=PEG-d-Alanine
28b=PEG-l-Alanine
30b:=PEG d-Ala-taxol ester
30c:=PEG l-Ala-taxol ester